CLAIMS

We claim:

1. A method of determining the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, an amount of parent bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample, said amount of parent bacteriophage being less than the threshold amount of bacteriophage capable of being detected in said bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target microorganism to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample; and

(c) assaying said bacteriophage exposed sample to detect the presence or absence of said bacteriophage or said biological substance associated with said bacteriophage to determine the presence or absence of said target microorganism.

2. A method as in claim 1 wherein said microorganism is a bacterium and said assaying comprises detecting said bacteriophage or a biological substance associated with said bacteriophage as an indication of the presence of said target bacterium in said sample.

3. A method as in claim 2 wherein said providing comprises providing conditions sufficient to cause said bacteriophage to multiply sufficiently to burst said target bacterium.

4. A method as in claim 1 wherein said assaying comprises applying said bacteriophage exposed sample to a lateral flow strip.

5. A method as in claim 1 wherein said assaying comprises complexing said bacteriophage or a biological substance associated with said bacteriophage with a colored bead.

6. A method as in claim 1 wherein said assaying comprises permitting an antibody to attach to said bacteriophage or a biological substance associated with said bacteriophage.

7. A method as in claim 1 wherein said sample is a fluid and said combining comprises pouring said sample into a container containing said parent bacteriophage.

8. A method as in claim 1 wherein said assaying comprises applying said bacteriophage exposed sample to a SILAS surface.

9. A method as in claim 8 wherein said assaying comprises contacting an enzyme to said SILAS surface after said applying.

10. A method as in claim 1 wherein said assaying comprises utilizing a mass spectrometer.

11. A method as in claim 10 wherein said utilizing comprises utilizing a MALDI mass spectrometer.

12. A method as in claim 10 wherein said utilizing comprises preparing a spectrometer sample using magnetic beads.

13. A method as in claim 1 wherein said assaying comprises exposing said sample to a laser beam.

14. A method as in claim 1 wherein said providing comprises lysing said microorganism prior to said assaying.

15. A method as in claim 14 wherein said lysing comprises permitting said bacteriophage to burst said microorganism.

16. A method as in claim 14 wherein said lysing comprises adding a microbial lysozyme to said bacteriophage exposed sample.

17. A method as in claim 14 where said lysing comprises a method selected from the group consisting of: adding chloroform to said bacteriophage exposed sample; treating said bacteriophage exposed sample with acid; and physically processing said bacteriophage exposed sample.

18. A method as in claim 14 wherein said providing further comprises dissociating said bacteriophage.

19. A method as in claim 18 wherein said dissociating comprises adding a dissociating agent to said bacteriophage exposed sample.

20. A method as in claim 19 wherein said adding comprises adding a substance selected from the group consisting of: acid, urea, denaturing agents, and

enzymes.

21. A method as in claim 1 wherein said providing comprises dissociating said bacteriophage.

22. A method as in claim 21 wherein said dissociating comprises adding a dissociating agent to said bacteriophage exposed sample.

23. A method as in claim 22 wherein said adding a dissociating agent comprises adding a substance selected from the group consisting of: acid, urea, denaturing agents, and enzymes.

24. A method as in claim 1 wherein said combining includes tagging said parent bacteriophage.

25. A method as in claim 24 wherein said tagging comprises a process selected from the group consisting of: biotinylating said parent bacteriophage; and attaching said parent bacteriophage to a physical substrate.

26. A method as in claim 24 and further comprising segregating said tagged bacteriophage from said test sample prior to said assaying.

27. A method as in claim 26 wherein said segregating comprises extracting said tagged bacteriophage from said test sample.

28. A method as in claim 26 wherein said tagging comprises biotinylating said parent bacteriophage and said segregating comprises attracting said biotinylated bacteriophage to streptavidin.

29. A method as in claim 28 wherein:

said assaying includes: providing a lateral flow strip having a sample application pad and a detection line; and applying said bacteriophage exposed sample to said sample application pad; and

said segregating comprises binding said biotinylated parent bacteriophage to a streptavidin coated portion of said lateral strip prior to said detection line.

30. A method as in claim 26 wherein said tagging comprises attaching said parent bacteriophage to a physical substrate and said segregating comprises isolating said physical substrate from said bacteriophage to be detected in said sample.

31. A method as in claim 30 wherein said isolating comprises removing said physical substrate from said test sample.

32. A method as in claim 1 wherein said bacteriophage is genetically modified.

33. A method as in claim 32 wherein said bacteriophage is genetically modified to enhance a desirable property of the infection process.

34. A method as in claim 32 wherein said bacteriophage is genetically modified to over-express a detectable biomarker.

35. A method as in claim 32 wherein said bacteriophage is genetically modified to express an enzyme.

36. A method as in claim 32 wherein said bacteriophage is genetically modified to express a target on the capsid protein.

37. A method of determining the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, an amount of parent bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target microorganism to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample; and

(c) applying said bacteriophage exposed sample to a lateral flow strip to determine the presence or absence of said target microorganism.

38. A method as in claim 37 wherein said microorganism is a bacterium and said assaying comprises detecting said bacteriophage or a biological substance associated with said bacteriophage as an indication of the presence of said target bacterium in said sample.

39. A method as in claim 38 wherein said providing comprises providing conditions sufficient to cause said bacteriophage to multiply sufficiently to burst said target bacterium.

40. A method of determining the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, an amount of parent bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target microorganism to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample; and

(c) applying said bacteriophage exposed sample to a SILAS surface to determine the presence or absence of said target microorganism.

41. A method as in claim 40 wherein said microorganism is a bacterium and said assaying comprises detecting said bacteriophage or a biological substance associated with said bacteriophage as an indication of the presence of said target bacterium in said sample.

42. A method as in claim 41 wherein said providing comprises providing conditions sufficient to cause said bacteriophage to multiply sufficiently to burst said target bacterium.

43. A method of determining the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, parent bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target microorganism to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample;

(c) obtaining a mass spectrum of said sample utilizing a matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOF-MS);

(d) utilizing said mass spectrum to determine the presence or absence of either said bacteriophage or a biological substance associated with said bacteriophage as an indication of the presence or absence of said target

microorganism.

44. A method as in claim 43 wherein said obtaining comprises concentrating said bacteriophage or said a biological substance associated with said bacteriophage exposed sample.

45. A method as in claim 44 wherein said concentrating comprises forming a complex of said bacteriophage or said a biological substance associated with said bacteriophage and magnetic beads and segregating said complex with a magnet.

46. A method of determining the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) without destroying, removing, neutralizing, or inactivating extracellular bacteriophage in said bacteriophage exposed sample, providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample; and

(c) assaying said bacteriophage exposed sample to detect the presence or absence of said bacteriophage or said biological substance associated with said bacteriophage to determine the presence or absence of said target microorganism.

47. A method as in claim 46 wherein said microorganism is a bacterium and said assaying comprises detecting said bacteriophage or a biological substance associated with said bacteriophage as an indication of the presence of said target bacterium in said sample.

48. A method as in claim 47 wherein said providing comprises providing conditions sufficient to cause said bacteriophage to multiply sufficiently to burst said target bacterium.

49. A method as in claim 46 wherein said assaying comprises providing a reference indicating an assay result if no target microorganism are present in said sample and comparing a corresponding result from said bacteriophage exposed sample to said reference result.

50. A method as in claim 46 wherein said providing comprises lysing said microorganism prior to said assaying.

51. A method as in claim 46 wherein said providing comprises dissociating said bacteriophage.

52. A method as in claim 46 wherein said combining includes tagging said parent bacteriophage.

53. A method of detecting the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target to create a detectable amount of a capsid protein associated with said bacteriophage in said bacteriophage exposed sample; and

(c) assaying said bacteriophage exposed sample to determine the presence or absence of said capsid protein associated with said bacteriophage as an indication of the presence or absence of said target microorganism.

54. A method as in claim 53 wherein said microorganism is a bacterium and said assaying comprises detecting said capsid protein as an indication of the presence of said target bacterium in said sample.

55. A method as in claim 53 wherein said providing comprises actively lysing said microorganism prior to said assaying.

56. A method as in claim 55 wherein said actively lysing comprises a method selected from the group consisting of: adding chloroform to said bacteriophage exposed sample; treating said bacteriophage exposed sample with acid; and physically processing said bacteriophage exposed sample.

57. A method as in claim 53 wherein said providing further comprises dissociating said bacteriophage.

58. A method as in claim 57 wherein said dissociating comprises adding a dissociating agent to said bacteriophage exposed sample.

59. A method as in claim 53 wherein said combining comprises tagging said

parent bacteriophage.

60. A method as in claim 59 wherein said assaying comprises removing said tagged parent bacteriophage from said bacteriophage exposed sample.

61. A method as in claim 53 wherein said combining includes tagging the capsid protein of said parent bacteriophage.

62. A method as in claim 53 wherein said assaying comprises providing a reference indicating an assay result if said target microorganism are not present in said sample and comparing a corresponding result from said bacteriophage exposed sample to said reference result.

63. A method of detecting the presence or absence of target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, parent bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to: allow said bacteriophage to infect said target microorganism and multiply in said target microorganism to create progeny bacteriophage; and produce a dissociated bacteriophage substance accessible to an assay; and

(d) assaying said bacteriophage exposed sample to determine the presence or absence of said bacteriophage substance as an indication of the presence or absence of said target microorganism in said sample.

64. A method as in claim 63 wherein said microorganism is a bacterium and said assaying comprises detecting said bacteriophage substance as an indication of the presence of said target bacterium in said sample.

65. A method as in claim 63 wherein said bacteriophage substance is a capsid protein.

66. A method as in claim 63 wherein said providing comprises lysing said microorganism to release said bacteriophage.

67. A method as in claim 66 wherein said lysing comprises adding a microbial lysozyme to said bacteriophage exposed sample.

68. A method as in claim 66 where said lysing comprises a method selected from the group consisting of: adding chloroform to said bacteriophage exposed

sample; treating said bacteriophage exposed sample with acid; and physically processing said bacteriophage exposed sample.

69. A method as in claim 63 wherein said dissociating comprises adding a dissociating agent to said bacteriophage exposed sample.

70. A method as in claim 69 wherein said adding comprises adding a substance selected from the group consisting of: acid, urea, denaturing agents, and enzymes.

71. A method as in claim 63 wherein said combining comprises tagging said parent bacteriophage and said providing includes segregating said tagged parent bacteriophage and then dissociating said bacteriophage to produce said dissociated bacteriophage substance.

72. A method as in claim 71 wherein said tagging comprises a process selected from the group consisting of: biotinylating said parent bacteriophage; and attaching said parent bacteriophage to a physical substrate.

73. A method as in claim 63 wherein said assaying comprises applying said bacteriophage exposed sample to a lateral flow strip.

74. A method of determining the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) tagging a sample of bacteriophage capable of infecting said target microorganism;

(b) combining said tagged bacteriophage with said sample to create a bacteriophage exposed sample;

(c) providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target microorganism to create progeny bacteriophage;

(d) segregating said tagged bacteriophage from said progeny bacteriophage; and

(c) assaying said bacteriophage exposed sample to detect the presence or absence of said progeny bacteriophage or a biological substance associated with said progeny bacteriophage to determine the presence or absence of said target microorganism.

75. A method as in claim 74 wherein said tagging comprises a process selected from the group consisting of: biotinylating said parent bacteriophage; and attaching said parent bacteriophage to a physical substrate.

76. A method as in claim 74 wherein said tagging comprises biotinylating said parent bacteriophage and said segregating comprises attracting said biotinylated bacteriophage to streptavidin.

77. A method as in claim 76 wherein:

said assaying includes: providing a lateral flow strip having a sample application pad and a detection line; and applying said bacteriophage exposed sample to said sample application pad; and

said segregating comprises binding said biotinylated parent bacteriophage to a streptavidin coated portion of said lateral strip prior to said detection line.

78. A method as in claim 74 wherein said tagging comprises attaching said parent bacteriophage to a physical substrate and said segregating comprises isolating said physical substrate from said bacteriophage to be detected in said sample.

79. A method as in claim 74 wherein said segregating comprises removing said tagged bacteriophage from said test sample using magnetic beads.

80. A method of determining the resistance or susceptibility of a target microorganism to an antibiotic, said method comprising:

(a) providing a sample containing said target microorganism;

(b) dividing said sample into a first sample and a second sample;

(c) adding said antibiotic to said second sample;

(d) combining each of said first and second samples with a bacteriophage capable of infecting said target microorganism to create a first bacteriophage exposed sample and a second bacteriophage exposed sample;

(e) providing conditions to said bacteriophage exposed samples sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target microorganism to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample;

(f) assaying said bacteriophage exposed samples to detect the presence

or absence of said bacteriophage or said biological substance associated with said bacteriophage to determine the presence or absence of said target microorganism in said first and second samples; and

(g) comparing said results of said assaying for said first and second samples to determine said resistance or susceptibility of said target microorganism to said one or more antibiotics.

81. A method as in claim 80 wherein said assaying comprises applying said first sample to a first lateral flow strip and applying said second sample to a second lateral flow strip.

82. A method as in claim 80 wherein said assaying comprises applying said first sample to a first SILAS surface and applying said second sample to a second SILAS surface.

83. A method as in claim 80 wherein said adding comprises adding a plurality of said antibiotics.

84. A method of determining the presence or absence of a target microorganism in a sample to be tested, said method comprising:

(a) combining with said sample, a parent bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to allow said bacteriophage to infect said target microorganism and to multiply in said target microorganism to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample;

(c) applying said bacteriophage exposed sample to a substrate at least a portion of which changes color if either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample is present; and

d) determining the presence or absence of said color change as an indication of the presence of absence of said target microorganism.

85. A method as in claim 84 wherein said applying comprises applying said bacteriophage exposed sample to a lateral flow strip.

86. A method as in claim 84 wherein said applying comprises applying said bacteriophage exposed sample to a SILAS surface.

87. A method of manufacturing a microbial test substrate, said method comprising:

providing a substrate and a biological material capable of attaching to a bacteriophage or a biological substance associated with said bacteriophage;

forming a line of said biological material on said substrate; and

cutting said substrate in a direction essentially perpendicular to said line to form said test substrate.

88. A method as in claim 87 wherein said substrate is a porous membrane.

89. A method as in claim 87 wherein said biological material is an antibody.

90. A method as in claim 87 wherein said providing comprises providing a first biological material and a second biological material, and said forming comprises forming a first line with said first biological material and a second line with said second biological material, with said first line and said second line being substantially parallel.

91. A method as in claim 90 wherein said providing further comprises providing a third biological material and said forming comprises forming a third line with said third biological material, said third line essentially parallel to first and second lines.

92. A method of manufacturing a bacteriological test substrate, said method comprising:

providing a substrate;

forming an optical coating on said substrate; and

securing a biological material on said optical coating, said biological material capable of attaching to a bacteriophage or a biological substance associated with said bacteriophage.

93. A method as in claim 92 wherein said securing comprises applying an attachment polymer to said optical coating and depositing said biological material on said attachment polymer.

94. A method as in claim 92 wherein said biological material is an antibody.

95. A method of detecting the presence or absence of target microorganism

in a sample to be tested, said method comprising:

(a) combining with said sample, parent bacteriophage capable of infecting said target microorganism to create a bacteriophage exposed sample;

(b) providing conditions to said bacteriophage exposed sample sufficient to: allow said bacteriophage to infect said target microorganism and multiply in said target microorganism to create a detectable amount of either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample;

(c) actively lysing said microorganism; and

(d) assaying said bacteriophage exposed sample to detect the presence or absence of said bacteriophage or said biological substance associated with said bacteriophage to determine the presence or absence of said target microorganism.

96. A method as in claim 1 wherein said target microorganism is a bacterium and said assaying comprises detecting said bacteriophage or a biological substance associated with said bacteriophage as an indication of the presence of said target bacterium in said sample.

97. A method as in claim 95 wherein said biological substance is a capsid protein.

98. A method as in claim 95 wherein said actively lysing comprises adding a microbial lysozyme to said bacteriophage exposed sample.

99. A method as in claim 95 where said actively lysing comprises a method selected from the group consisting of: adding chloroform to said bacteriophage exposed sample; treating said bacteriophage exposed sample with acid; and physically processing said bacteriophage exposed sample.

100. Apparatus for detecting a target microorganism, said apparatus comprising:

a substrate;

an immobilization zone on said substrate, said immobilization zone including an immobilization agent designed to immobilize a bacteriophage or a biological substance associated with a bacteriophage; and

a color moderator designed to interact with said a bacteriophage or a biological

substance associated with a bacteriophage, whereby the presence of said bacteriophage or said biological substance associated with a bacteriophage causes said immobilization zone to change color.

101. Apparatus as in claim 100 wherein said immobilization zone comprises antibodies.

102. Apparatus as in claim 101 wherein said color moderator comprises colored beads.

103. Apparatus as in claim 101 wherein said color moderator comprises a reacting agent and an enzyme which form a precipitant upon reacting.

104. Apparatus as in claim 103 wherein said reacting agent comprises a material selected from the group consisting of: horseradish peroxidase (HRP) and alkaline phosphatase, and said enzyme comprises 3,3',5,5' tetramethylbenzidine (TMB).

105. Apparatus as in claim 100 wherein said substrate comprises a lateral flow strip.

106. Apparatus as in claim 100 wherein said substrate comprises a SILAS surface.

107. Apparatus as in claim 100 wherein said microorganism is a bacterium.

108. A kit for determining the presence or absence of a target microorganism in a sample to be tested, said kit comprising: a first container containing a bacteriophage capable of infecting said target microorganism; and

a substrate at least a portion of which changes color if either said bacteriophage or a biological substance associated with said bacteriophage in said bacteriophage exposed sample is present.

109. A kit as in claim 108 and further comprising a second container containing a buffer solution.

110. A kit as in claim 108 wherein said substrate comprises a lateral flow strip.

111. A kit as in claim 108 wherein said substrate comprises a SILAS surface.

112. A kit as in claim 108 wherein said first container includes a dropper designed to release drops of a predetermined size.

113. A kit as in claim 108 wherein said target microorganism is a bacterium.

114. A kit as in claim 108 and further comprising directions for determining the presence or absence of a target microorganism in a sample to be tested using said kit.

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